Cystatin C-based calculation of glomerular filtration rate in kidney transplant recipients

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Cystatin C (Cys C) has been shown to be an alternative marker of renal function. However, estimation of the glomerular filtration rate (GFR) based on Cys C has received little attention. Recently, several Cys C-based equations were developed in different patient cohorts. To date, the benefit of a Cys C-based GFR calculation in patients after renal transplantation (RTx) remains to be elucidated. We compared the diagnostic accuracy of three Cys C-based formulae (Larsson, Hoek, Filler which used an immunonephelometric method) with the results of the Modification of Diet in Renal Disease (MDRD) formula. GFR was measured by means of technetium-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) clearance in 108 consecutive patients after RTx. Correlation coefficients of all calculated GFR estimates with the true GFR were high but did not differ significantly from one another (0.83–0.87). The MDRD and Filler equations overestimated GFR significantly, whereas the Larsson equation significantly underestimated GFR. Bias of the Hoek formula was negligible. Precision of the Hoek (8.9 ml/min/1.73 m^2) and Larsson equations (9.6 ml/min/1.73 m^2) were significantly better than MDRD equations (11.4 ml/min/1.73 m^2; $P \leqslant 0.035$ each). Accuracy within 30% of real GFR was 67.0 and 65.1% for the MDRD and Filler formulae, and 77.1% for the Larsson and Hoek formulae, respectively. Accuracy within 50% of true GFR for the Hoek formula (97.2%) was better than for the MDRD equations (85.3%). Cys C-based formulae may provide a better diagnostic performance than creatinine-based equations in GFR calculation after RTx.

Kidney International (2006) **70,** 204–210. doi:10.1038/sj.ki.5001502; published online 24 May 2006

KEYWORDS: cystatin C equation; glomerular filtration rate; renal transplantation; MDRD

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Received 30 March 2005; revised 5 February 2006; accepted 22 February 2006; published online 24 May 2006

After kidney transplantation the glomerular filtration rate (GFR) is considered to be the best index for monitoring graft function. Renal clearance of exogenous markers, such as inulin, technetium-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA), ^{51}Cr–EDTA, and ^{125}I-Iothalamate are accepted as the best measure to determine GFR. However, such procedures are invasive, time consuming, expensive and hence not ideal for clinical practice or large-volume clinical research.

Therefore, GFR calculation is recommended.[1] Unfortunately, the most commonly used Cockroft and Gault formula (C&G) as well as several other equations have been reported to be inaccurate in patients after renal transplantation (RTx).[2,3] As a consequence, interest has arisen in alternative endogenous markers of renal function. In a recent meta analysis, cystatin C (Cys C), a 13.3-kDa protein, was evaluated in patients with various renal diseases and found to be superior to creatinine.[4] In patients after RTx, Cys C performs at least as well as creatinine as a kidney function marker.[5–7]

Recently, three formulae using Cys C serum levels instead of creatinine as an endogenous marker were suggested for calculation of GFR in adult patients as well as in children with various renal diseases.

In all three formulae, the identical immunonephelometric Cys C method on a Dade Behring nephelometer was applied. We evaluated these three formulae in comparison to the simplified Modification of Diet in Renal Disease (MDRD) formula[8] in an unselected consecutive cohort of renal transplant recipients who had their GFR determined by a gold standard method.

RESULTS

In this cohort, the mean creatinine concentration was 159 (95% confidence interval (CI) 144–174) μmol/l. The mean Cys C concentration was 2.24 (95% CI 2.03–2.45) mg/l.

Mean GFR measured by ^{99m}Tc-DTPA was 39.5 (95% CI 36.4–42.6) ml/min/1.73 m^2. GFR estimates based on the MDRD, Larsson and Filler equations differed significantly from ^{99m}Tc-DTPA clearance ($P < 0.001$ for all). In contrast, results from the Hoek formula did not differ from true GFR ($P = 0.5$). All tests describing GFR correlated well with the

Table 1 | Statistical data from the different GFR estimates

	Median estimates (ml/min/1.73 m^2)	Range (ml/min/1.73 m^2)	Correlation coefficient (r)	Median difference (ml/min/1.73 m^2)	Median absolute difference (ml/min/1.73 m^2)	Median % difference (%)	10% (95% CI) (%)	Accuracy within 30% (95% CI) (%)	50% (95% CI) (%)
DTPA	37.0	11.8–82.9							
MDRD	46.2	10.0–115	0.832	+6.75	7.90	+20.3	24.8 (17.6–33.7)	67.0 (57.7–75.1)	85.3 (77.4–90.9)
Larsson	33.2	7.78–104	0.859	−4.78	6.00	−14.7	20.2 (13.7–28.8)	77.1 (68.3–84.0)	95.4 (89.5–98.3)
Hoek	36.9	8.72–97.4	0.865	−1.50	7.40	−4.40	33.0 (24.9–42.4)	77.1 (68.3–84.0)	97.2 (91.9–99.4)
Filler	43.3	11.9–119	0.863	+3.95	6.30	+12.4	28.4 (20.8–37.6)	65.1 (55.8–73.5)	87.2 (79.5–92.3)

(95% CI)=95% confidence interval; DTPA: diethylenetriamine pentaacetic acid; MDRD: Modification of Diet in Renal Disease formula; GFR: glomerular filtration rate.

measures of the ^{99m}Tc-DTPA clearance ($P<0.0001$ for all). No significant differences in correlation coefficients were found between the applied equations (see Table 1).

A mean overestimation of 7.92 (95% CI 5.73–10.1) and 6.72 (95% CI 4.62–8.82) ml/min/1.73 m^2 was found for the MDRD and Filler equations, respectively. In contrast, the Larsson formula underestimated the true GFR by −3.20 (95% CI −5.03 −1.36) ml/min/1.73 m^2. Furthermore, the results of the Hoek formula were very similar to the true GFR −0.58 (95% CI −2.29 −1.14) ml/min/1.73 m^2. The mean difference of the estimates derived from the Hoek formula and the true GFR was significantly lower than the results of all other formulae ($P<0.0001$ for all). The bias of the Larsson equation was significantly different from the MDRD and the Filler equation ($P<0.0001$ for both). Bias of the MDRD equation did not differ significantly from the Filler equation ($P=0.29$).

Root mean square error (RMSE) was used as a measure of precision: the higher the RMSE the lower the precision. The MDRD formula (11.43 ml/min/1.73 m^2) was significantly worse compared to the Larsson GFR (9.59 ml/min/1.73 m^2, $P=0.035$; F-statistics: 1.4205) and the Hoek GFR (8.94 ml/min/1.73 m^2, $P=0.006$; F-statistics: 1.6346). No differences were found between precision of the Filler (10.95 ml/min/1.73 m^2) and the MDRD formula ($P=0.33$).

F-statistics showed no differences between the Hoek and Larsson GFR ($P=0.23$) and between Filler and Larsson equation ($P=0.09$). However, Hoek formula was more precise than the Filler equation ($P=0.019$; F-statistics: 1.5002).

As suggested by clinical practical guidelines of the National Kidney Foundation, the analysis of a new GFR equation should include the proportion of the GFR estimates which are within 30 and 50% of measured GFR. Additionally, we provide the data for a 10% cutoff to test whether Cys C-based equations can sufficiently replace gold standard measurements.[9] Results are given in Figure 1.

When we compared the 95% CI of the different equations we found that the 95% CI of the Hoek and MDRD equation did not overlap for the 50% accuracy indicating a significant higher accuracy of the Hoek equation.

Bland and Altman plots, a graphical method to demonstrate the magnitude and consistency of the differences between calculated and measured values, are presented in Figure 2a–d.

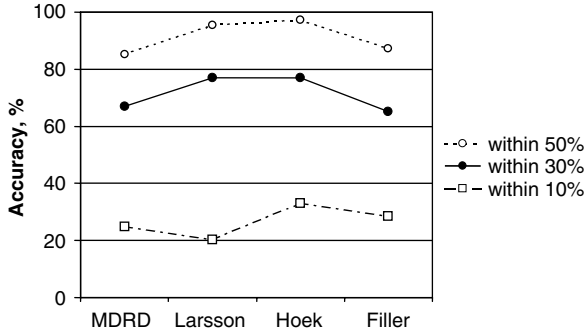

Figure 1 | Accuracy of different GFR estimates expressed as the percentage of estimates within 10, 30, and 50% of the true GFR. Highest accuracy was found for the Hoek equation.

The graphical technique demonstrates the span between +1.96 s.d. and −1.96 s.d. of the mean difference between the calculated and the true GFR.

The limits of agreement estimated for the Hoek equation are 18 ml/min/1.73 m^2 below or 17 ml/min/1.73 m^2 above the true GFR. Similarly, the Larsson equation showed a span between −22 and 16 ml/min/1.73 m^2. Comparing the range between the limits of agreement of the Hoek and the Larsson formula (35 and 38 ml/min/1.73 m^2, respectively) with the MDRD, we found a considerably higher distance between both limits for the MDRD estimates (45 ml/min/1.73 m^2) (Table 2).

Two further questions were aimed in our analysis: First, to identify the impact of higher steroid dosages on the performance of Cys C-based equations. Second, we analyzed the diagnostic performance of the Cys C-based formulae in patients with CKD stages 4 and 5 since numbers of patients with chronic renal allograft nephropathy and long-term survival after transplantation increase. In the first analysis, we compared a subgroup of patients with 10 mg or more prednisone per day ($n=14$, group A) with the majority of our patients who received 5 mg or less prednisone per day ($n=87$, group B) (Table 3).

Both Cys C-based GFR estimations which showed results similar to GFR in group B were found to considerably underestimate true GFR in group A. The Filler equation overestimated GFR in the low-dose group, but did not differ

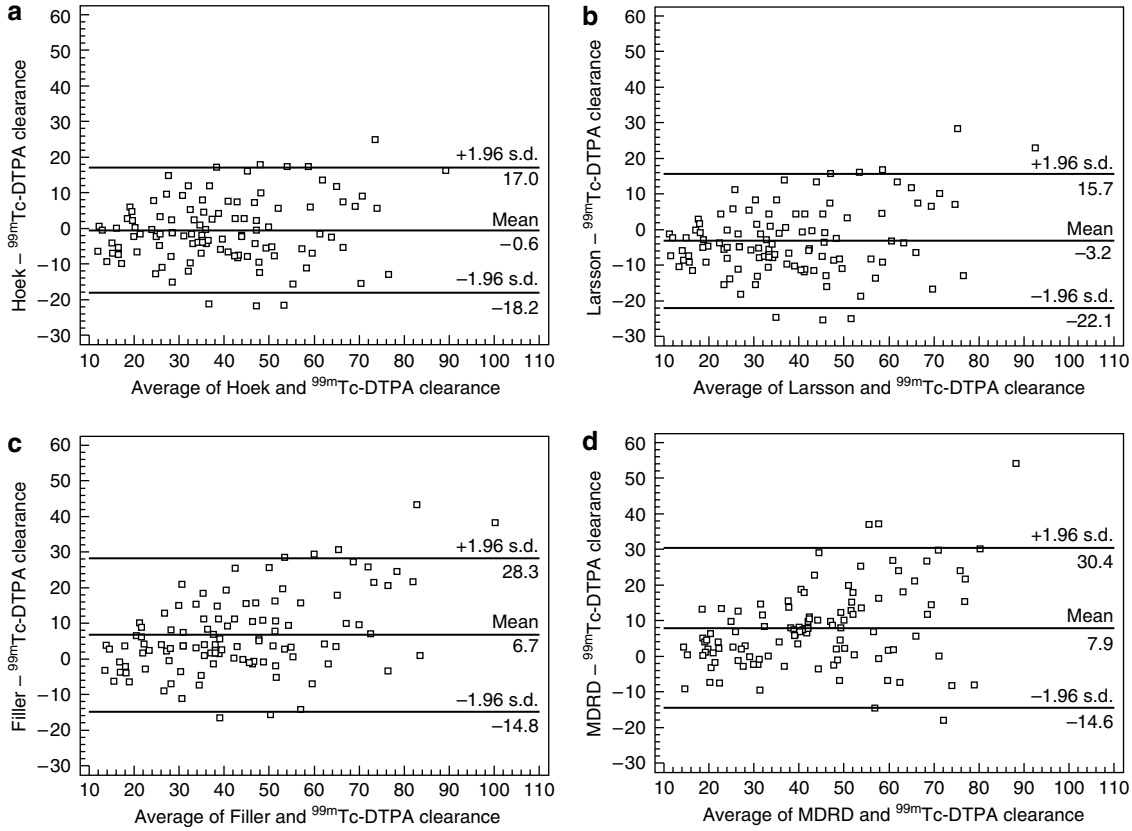

Figure 2 | Bland and Altman analysis of GFR estimates in 108 patients. In this analysis, the differences between two methods are plotted against the true GFR for each individual patient. The mean difference is indicated by the line, limits of agreement are indicated by the dotted lines. (**a**) Shows the results for the Hoek equation and the GFR, (**b**) the results for the Larsson equation are given, (**c**) for the Filler equation, and (**d**) for the MDRD equation in, respectively. Data are given in ml/min/1.73 m^2.

Table 2 | Limits of agreement of GFR estimates

	Lower limit (95% CI) (ml/min/1.73 m^2)	Upper limit (95% CI) (ml/min/1.73 m^2)
MDRD	−14.6 (−16.8–12.4)	30.4 (28.2–32.6)
Larsson	−22.1 (−23.9–20.2)	15.7 (13.8–17.5)
Hoek	−18.2 (−19.9–16.5)	17.0 (15.3–18.7)
Filler	−14.8 (−16.9–12.8)	28.3 (26.2–30.4)

(95% CI)=95% confidence interval; MDRD: Modification of Diet in Renal Disease formula; GFR: glomerular filtration rate.

from true GFR in the subgroup of patients with 10 mg or more prednisone per day. The differences in bias were significant between groups A and B for all Cys C-based GFR estimations ($P<0.015$). Moreover, the precision of the Filler equation was significantly better in the 'high' prednisone dose group ($P=0.028$; F-statistics: 2.612).

On the contrary, in the 'low'-dose group, the accuracy within 50% of true GFR showed no overlap of the 95% CI when Hoek equation was compared with the MDRD and the Filler formulae. However, the Larsson formula showed a small overlap of the 95% CI with the MDRD and Filler equation. At the accuracy within 30 and 10% of true GFR, none of the Cys C-based GFR equations was significantly superior to the MDRD formula.

In the second subanalysis, we investigated the performance of the equations in 33 patients with a true GFR below 30 ml/min/1.73 m^2 (Table 4). We expected a better performance of the MDRD equation due to exponential increase of creatinine when GFR falls below 30 ml/min/1.73 m^2. However, Larsson and Hoek formulae were slightly better than MDRD with regard to correlation, bias, precision, and accuracy (n.s.).

DISCUSSION

In this study, the diagnostic performance of four methods of GFR estimation was tested against the gold standard-derived GFR. A combination of different statistical analyses was used to evaluate their particular performance in patients after kidney transplantation. We demonstrate that the diagnostic performance of Cys C-based formulas that are easy to perform are more accurate than or at least equal to the four different variables containing MDRD formula.

It is well known that serum creatinine concentrations are influenced by muscle mass, dietary protein intake, sex and age,[10] thus limiting the precision of creatinine-based methods. Furthermore, in patients with reduced GFR, tubular secretion of creatinine increases.[11] Consequently, creatinine-based GFR estimates like the Cockroft and Gault equation overestimate true GFR.[12]

Table 3 | Subanalysis of different GFR estimates for patients receiving different corticosteroid dosages

	Median estimates (ml/min/1.73 m^2)	Range (ml/min/1.73 m^2)	Correlation coefficient (r)	Mean difference (ml/min/1.73 m^2)	RMSE (ml/min/1.73 m^2)	10% (95% CI) (%)	Accuracy within 30% (95% CI) (%)	50% (95% CI) (%)
Group A								
DTPA	38.0	16.0–58.0						
MDRD	43.8	16.7–74.2	0.816	6.84	8.57	14.3 (2.91–41.5)	57.1 (32.8–78.9)	71.4 (45.2–88.9)
Larsson	28.9	8.10–43.7	0.853	−8.75	6.32	21.4 (7.01–48.6)	64.3 (38.8–84.0)	92.9 (66.8–99.9)
Hoek	32.6	9.20–46.7	0.860	−5.66	6.29	35.7 (16.4–61.6)	42.9 (21.6–67.7)	64.3 (38.8–84.0)
Filler	38.2	12.3–55.2	0.857	0.05	6.92	14.3 (2.91–41.5)	50.0 (27.0–73.4)	71.4 (45.2–88.9)
Group B								
DTPA	37.0	11.8–82.9						
MDRD	48.0	10.0–115	0.831	8.18	12.0	26.4 (18.3–36.7)	63.2 (52.8–72.7)	85.1 (76.0–91.2)
Larsson	34.3	7.78–104	0.865	−1.91	9.85	19.5 (12.5–29.2)	79.3 (69.6–86.6)	96.6 (90.0–99.3)
Hoek	38.0	8.72–97.4	0.871	0.59	9.13	32.2 (23.3–42.6)	78.2 (68.4–85.7)	97.7 (91.6–99.9)
Filler	44.6	11.9–119	0.868	8.28	11.2	27.6 (19.3–37.9)	58.6 (48.2–68.4)	83.9 (74.7–90.3)

(95% CI)=95% confidence interval; DTPA: diethylenetriamine pentaacetic acid; GFR: glomerular filtration rate; MDRD: Modification of Diet in Renal Disease formula; RMSE: root mean square error.
Group A: patients with $\geqslant$10 mg prednisone per day (n=14).
Group B: patients with $\leqslant$5 mg prednisone per day (n=87).

Table 4 | Subanalysis of different GFR estimates for patients with DTPA-GFR $<$30 ml/min/1.73m^2

	Median estimates (ml/min/1.73 m^2)	Range (ml/min/1.73 m^2)	Correlation coefficient (r)	Mean difference (ml/min/1.73 m^2)	RMSE (ml/min/1.73 m^2)	10% (95% CI) (%)	Accuracy within 30% (95% CI) (%)	50% (95% CI) (%)
DTPA	21.0	11.8–29.8						
MDRD	24.0	10.0–58.9	0.588	+4.40	7.61	27.3 (15.0–44.5)	57.6 (40.9–72.9)	81.8 (65.4–91.9)
Larsson	18.8	7.78–43.7	0.698	−1.95	6.10	18.2 (8.32–34.9)	66.7 (49.6–80.4)	90.9 (75.8–97.7)
Hoek	21.9	8.72–46.9	0.704	+0.86	6.62	33.3 (19.8–50.6)	63.6 (46.7–78.0)	90.9 (75.8–97.7)
Filler	26.0	11.9–55.2	0.702	+5.29	7.64	12.1 (4.29–28.1)	57.6 (40.9–72.9)	75.8 (58.9–87.5)

(95% CI)=95% confidence interval; DTPA: diethylenetriamine pentaacetic acid; MDRD: Modification of Diet in Renal Disease formula; RMSE: root mean square error.

The simplified MDRD formula is recommended by the National Kidney Foundation to estimate GFR in cohorts similar to the MDRD participants.[13] However, in cohorts dissimilar to the MDRD study group and especially in patients after kidney transplantation, the diagnostic performance of the MDRD formula was suboptimal indicating that gold standard clearance may still be required.[2] Despite the fact that we recently suggested the so-called MDRD6 formula as the best-performing GFR estimation in patients after kidney transplantation[14] we preferred the simplified MDRD as a comparator for the Cys C-based equations in this study. This is based on the fact that our promising results of the MDRD6 formula still warrant confirmation by other groups. Additionally, several publications recently demonstrated in patients after RTx that accuracy and precision of the simplified MDRD formula are better than that of the Cockcroft–Gault formula.[2,3,14] Thus, the simplified MDRD formula seems to be more suitable.

Even so, the question arises what may be better, the MDRD6 equation or Cys C-based formulae. In our present study the MDRD6 formula showed an accuracy within 50% of true GFR of 89.5%, which was inferior to the Cys C-based Larsson and Hoek formulae (data not shown).

Of the three tested Cys C-based equations the Filler formula did not differ from MDRD estimates with respect to correlation, bias, precision and accuracy.

Although the Filler formula was derived from a cohort where the ^{99m}Tc-DTPA clearance was performed – similar to our technique – using a single-injection technique and a multiple point blood sampling method, the estimates differed significantly from true GFR. Different patient characteristics may account for these divergences: Fillers cohort comprised children aged 1–18 years having a significant hyperfiltration with a mean GFR above 100 ml/min/1.73 m^2, which is more than twice the mean GFR in our study. Despite the fact that Cys C concentrations do not change after the first year of life, it is not known whether the same Cys C value reflects the same GFR over decades.

The Larsson formula had been evaluated in a cohort of 100 patients aged 4–92 years where GFR was determined by iohexol clearance, showing a GFR of 10–115 ml/min/1.73 m^2. No control group was included since two commercially available Cys C assays were compared.

In comparison to the Larsson formula, the Hoek equation which was derived by linear regression showed a better accuracy and precision.[15] The 146 participants of the

Hoek study differed from our cohort with respect to age, heterogeneity of renal function and the underlying renal diseases. Importantly, the Hoek study was the only one that used a continuous infusion technique as the gold standard method for GFR determination. This might be a possible explanation for the fact that the Hoek formula is more accurate and precise than the other formulae.

Despite the improved GFR estimation by the Hoek and Larsson formulae it should be noted that an ideal GFR equation as suggested by the National Kidney Foundation ought to cover 99% of all tests within 10% of true GFR.[9] Such an ambitious objective is highly recommended in patients with renal grafts. However, from this point of view all tested equations are far from being ideal. Yet, by time and with increasing knowledge of Cys C-derived GFR equations in RTx it may be possible to further improve the diagnostic performance of these equations. Nonetheless, we must bear in mind that currently, a gold standard clearance procedure cannot be replaced by these estimates.

Continuous low-dose steroid medication, as performed in our cohort, has been supposed to increase Cys C serum levels.[16] Based on this hypothesis, the possibly falsely elevated Cys C level resulted in an underestimation of GFR, which has recently been described in children.[17] This steroid-induced underestimation of GFR may be reflected by the negative bias of the Hoek and Larsson formulae. Although data on steroid effects remain inconsistent,[18] we performed a subanalysis comparing patients differing in prednisone dosages. Since the 'low'-dose subgroup comprised the majority of our cohort, these results did not differ from the entire study population. On the contrary, the 14 patients receiving 10 mg or more prednisone per day showed a considerable underestimation of GFR when Hoek and Larsson formulae were used.

However, as our study was not designed to perform such an analysis both subgroups had a different size and differed also in mean time since transplantation (group A: 4.3 vs group B: 91.1 months). Precision of the Filler formula was best in the 'high' steroid dosage group, however, we ought to keep in mind the small sample size. Therefore, these data have to be interpreted with caution as our conclusion warrants further confirmation by other investigators.

Besides corticosteroids immunosuppression with calcineurin inhibitors has also been proposed to influence Cys C serum levels.[7,19] Thus, we tested the hypothesis whether both of the applied calcineurin inhibitors affect the performance of the Cys C-based equations. However, neither bias, precision nor accuracy were different in patients receiving cyclosporine A or tacrolimus (data not shown).

Since creatinine increases exponentially when GFR drops below 30 ml/min/1.73 m^2, we performed a further subanalysis in patients with advanced renal failure and tested the diagnostic performance of the Cys C-based equations. Although the MDRD equation performed somewhat better than in the entire cohort, the Cys C-based Hoek and Larsson formulae remained superior, although these differences did not reach statistical significance. However, it should be stressed, that the 33 patients may not be enough to detect differences.

Most recently, White *et al.*[20] investigated Cys C-based equations in a cohort after kidney transplantation. In this study, the accuracy of the Filler formula was superior to the Hoek formula. Whereas in both studies, the Cys C-based equations by Hoek and Filler showed a higher precision than creatinine-based formulae, differences occurred with respect to bias. Similar to our results, the Filler formula calculated the highest GFR values of all tested Cys C-based equations. However, in this cohort, the bias of the Filler formula was smaller, whereas results of the Hoek and Larsson formulae underestimated true GFR to a higher degree. Since the true GFR was, on average, roughly 20 ml/min/1.73 m^2 higher than in our study, the different results may depend on dissimilar patient characteristics.

Finally, some limitations of our study should be mentioned: The impact of calibration of the serum creatinine assay on MDRD estimates has recently been emphasized.[21] The Jaffé method is known to detect non-creatinine chromogens also possibly resulting in an overestimation of creatinine concentration and consecutively in an underestimation of creatinine-based GFR estimates. To counteract this problem we used a kinetic modification of the Jaffé method.[22] Since we did not used the same MDRD study system (Beckman CX3) we cannot definitively exclude a possible bias. However, Hallan *et al.*[21] pointed out that the bias due to a missing calibration decreases as serum creatinine level increases. This is crucial since our cohort comprises a considerably percentage of patients with elevated creatinine.

Furthermore, the size of our study cohort was small in comparison to the number of participants of the MDRD study. However, the different Cys C-based formulae were derived from cohorts in dissimilar circumstances and paraphernalia with respect to staff, laboratory work, concomitant medication and reference standard clearance. Yet, these conditions do not facilitate a systemic bias and thus may rather support our conclusions.

Since our study contained 15 double-transplanted patients it could be argued that our cohort may be somewhat heterogeneous. Nevertheless, analysis of the study group without these patients did not alter the results (data not shown).

As mentioned above the Cys C-based formulae were originally tested in cohorts covering a wide range of GFR. In contrast to those our cohort comprises only patients with a relatively small range of kidney function. This may affect our results, especially with respect to the remarkably high calculated correlation coefficient.

Furthermore, it should be pointed out that all tested equations in this analysis were derived from studies that used the identical immunonephelometric assay for Cys C determination. Since turbidimetric immunoassays tend to produce higher values, our results may not be transferable to these assays.[23]

Despite these limitations our study shows the diagnostic equality of Cys C-based equations to the MDRD formula. Importantly, the Cys C-based Hoek formula was statistically better than the MDRD equation. However, since the accuracy within 30 and 50% of true GFR was enhanced merely by ~10% the clinical impact on daily practice remains to be clarified by further investigations. Nevertheless, we demonstrate that the recently proposed Cys C-based GFR formulae are appropriate for calculating GFR in patients after RTx. Since the overall performance of these formulae are remarkable even when low-dose prednisone medication ($\leqslant$5 mg/day) is administered, we conclude that Cys C-based equations might potentially improve the performance of prediction of GFR in RTx patients. However, further confirmation is warranted and currently, a reference method of GFR determination is required when GFR is an end point in clinical trials.

MATERIALS AND METHODS

Patients

One hundred and eight consecutive Caucasian patients (45 females, 63 males) with a mean age of 48.9 (95% CI 46.3–51.4) years underwent ^{99m}Tc-DTPA clearance measurements as part of clinical monitoring in the post-transplant period. This prospective study was approved by the local Ethics Committee and informed consent was obtained from all patients enrolled in the study.

The cohort included 12 patients after simultaneous kidney and pancreas transplantation and three patients after combined liver and kidney transplantation. 63 patients were treated with cyclosporin A and 43 patients received tacrolimus. Calcineurin inhibitor treatment was combined with mycophenolate-mofetil or azathioprine in 47 and three patients, respectively. One patient received sirolimus, mycophenolate-mofetil and corticosteroids, and another one sirolimus, tacrolimus and corticosteroids. All participants except two received corticosteroids. Mean corticosteroid dosage was 6 mg per day.

Patients included in the study were in 'steady state' conditions, which were defined as lack of increase or decrease of more than 15% of creatinine within 2 weeks before and after the investigation.

The mean time frame of investigation was 75.4 (95% CI 61.2–87.6) months after transplantation (range 3–240 months; 25 within 12 months after transplantation, 36 one to five years, 16 six to ten years, and 31 more than 10 years after RTx).

GFR, creatinine, and Cys C measurement

^{99m}Tc-DTPA –clearance was performed as single injection technique with a two point blood sampling according to the method of Russell *et al.*[24] In contrast to conventional one-compartment models this method is based on a two-compartment model and known to prohibit overestimation, which has been shown for one compartment models.[24] The results were corrected to the body surface area which was calculated using the 'Du Bois formula'.[25]

Serum creatinine and Cys C were measured from the same blood samples. Cys C in serum was analyzed by a fully automated latex-enhanced immunonephelometric method covering the range of 0.3–8 mg/l (N Latex Cys C Nephelometer II, Dade-Behring, Marburg, Germany). Serum creatinine was determined on the Dimension™ clinical chemistry system (Dade-Behring, Marburg, Germany) with a commercially available assay based on a modification of the kinetic Jaffé reaction reported by Larsen.[22] The sensitivities, intrassay, and interassay coefficients of both methods were as described elsewhere.[26]

GFR was estimated using the following formulae:

Hoek formula: $GFR = -4.32 + 80.35 \times 1/\text{Cys C}$[15]

Filler formula: $\log(GFR) = 1.962 + (1.123 * \log(1/\text{Cys C}))$[27]

Larsson formula: $GFR = 77.239 \times \text{Cys C}^{-1.2623}$[28]

Simplified MDRD (MDRD) $= 186 \times [\text{serum creatinine (mg/dl)}]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if patient is female}] \times [1.21 \text{ if patient is African-American}]$.[8]

Statistics

All results are given as mean plus (95% CI). Correlations between ^{99m}Tc-DTPA clearance and estimates of GFR were calculated by linear regression analysis (Pearson's correlation).

Bias was calculated by the mean difference between the true GFR (^{99m}Tc-DTPA clearance, serving as gold standard) and the estimated equation-based GFR. Pair-wise comparison of the mean difference was performed using paired *t*-test.

The precision of the estimates was expressed in terms of the RMSE as described elsewhere (RMSE; s.d. of the mean difference between real GFR and estimated GFR).[29]

The proportion of GFR estimates within 10, 30, and 50% deviation of the true GFR served as a measure of accuracy.[9]

Statistics were carried out using StatView™ (version 5.0 for Windows; SAS Institute Inc., Cary, NC, USA). Bland and Altman analyses of the GFR estimates and the true GFR were performed with Medcalc™ Software, Mariakerke, Belgium.[30] *P*-values below 0.05 were considered significant.

ACKNOWLEDGMENTS

We are grateful to Ms Lilly Rempel and Mrs Doris Ehlert for skillful technical assistance. We thank Ms Martina Schmidt and Ms Anke Carstensen for excellent technical assistance in performing the creatinine and cystatin C assays.

REFERENCES

1. Kasiske BL, Vazquez MA, Harmon WE *et al.* Recommendations for the outpatient surveillance of renal transplant recipients. American Society of Transplantation. *J Am Soc Nephrol* 2000; **11**(Suppl 15): S1–S86.
2. Gaspari F, Ferrari S, Stucchi N *et al.* Performance of different prediction equations for estimating renal function in kidney transplantation. *Am J Transplant* 2004; **4**: 1826–1835.
3. Mariat C, Alamartine E, Barthelemy JC *et al.* Assessing renal graft function in clinical trials: can tests predicting glomerular filtration rate substitute for a reference method? *Kidney Int* 2004; **65**: 289–297.
4. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; **40**: 221–226.
5. Risch L, Herklotz R, Blumberg A, Huber AR. Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem* 2001; **47**: 2055–2059.
6. Herget-Rosenthal S, Trabold S, Huesing J *et al.* Cystatin C – an accurate marker of glomerular filtration rate after renal transplantation? *Transplant Int* 2000; **13**: 285–289.
7. Pöge U, Stoschus B, Stoffel-Wagner B *et al.* Cystatin C as an endogenous marker of glomerular filtration rate in renal transplant patients. *Kidney Blood Press Res* 2003; **26**: 55–60.
8. Levey AS, Greene T, Kusek JW, Beck GJ. A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol* 2000; **11**: A0828.
9. K/DOQI clinical practice guidelines for chronic kidney disease. Evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1–S266.

10. Star R, Hostetter T, Hortin GL. New markers for kidney disease. *Clin Chem* 2002; **48**: 1375–1376.
11. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 1985; **28**: 830–838.
12. Kemperman FA, Krediet RT, Arisz L. Formula-derived prediction of the glomerular filtration rate from plasma creatinine concentration. *Nephron* 2002; **91**: 547–558.
13. Lewis J, Agodoa L, Cheek D *et al.* Comparison of cross-sectional renal function measurements in African Americans with hypertensive nephrosclerosis and of primary formulas to estimate glomerular filtration rate. *Am J Kidney Dis* 2001; **38**: 744–753.
14. Pöge U, Gerhardt T, Palmedo H *et al.* MDRD equations for estimation of GFR in renal transplant recipients. *Am J Transplant* 2005; **5**: 1306–1311.
15. Hoek FJ, Kemperman FA, Krediet RT. A comparison between cystatin C. plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol Dial Transplant* 2003; **18**: 2024–2031.
16. Risch L, Herklotz R, Blumberg A, Huber AR. Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem* 2001; **47**: 2055–2059.
17. Bokenkamp A, Domanetzki M, Zinck R *et al.* Cystatin C serum concentrations underestimate glomerular filtration rate in renal transplant recipients. *Clin Chem* 1999; **45**: 1866–1868.
18. Bokenkamp A, van Wijk JA, Lentze MJ, Stoffel-Wagner B. Effect of corticosteroid therapy on serum cystatin C and beta2-microglobulin concentrations. *Clin Chem* 2002; **48**: 1123–1126.
19. Cimerman N, Brguljan PM, Krasovec M *et al.* Serum cystatin C, a potent inhibitor of cysteine proteinases, is elevated in asthmatic patients. *Clin Chim Acta* 2000; **300**: 83–95.
20. White C, Akbari A, Hussain N *et al.* Estimating glomerular filtration rate in kidney transplantation: a comparison between serum creatinine and cystatin C-based methods. *J Am Soc Nephrol* 2005; **16**: 3763–3770.
21. Hallan S, Asberg A, Lindberg M, Johnsen H. Validation of the modification of diet in renal disease formula for estimating GFR with special emphasis on calibration of the serum creatinine assay. *Am J Kidney Dis* 2004; **44**: 84–93.
22. Larsen K. Creatinine assay by a reaction-kinetic principle. *Clin Chim Acta* 1972; **41**: 209–217.
23. Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem* 2002; **48**: 699–707.
24. Russell CD, Bischoff PG, Kontzen FN *et al.* Measurement of glomerular filtration rate: single injection plasma clearance method without urine collection. *J Nucl Med* 1985; **26**: 1243–1247.
25. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. *Nutrition* 1989; **5**: 303–311.
26. Woitas RP, Stoffel-Wagner B, Flommersfeld S *et al.* Correlation of serum concentrations of cystatin C and creatinine to inulin clearance in liver cirrhosis. *Clin Chem* 2000; **46**: 712–715.
27. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? *Pediatr Nephrol* 2003; **18**: 981–985.
28. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in ml/min from plasma cystatin C values in mg/l. *Scand J Clin Lab Invest* 2004; **64**: 25–30.
29. Walser M. Assessing renal function from creatinine measurements in adults with chronic renal failure. *Am J Kidney Dis* 1998; **32**: 23–31.
30. Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995; **346**: 1085–1087.